Adventitious shoot regeneration of Cuzi scarlet eggplant (*Solanum aethiopicum* L.) in tissue culture

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Abstract

Cuzi scarlet eggplant (*Solanum aethiopicum* L.) is a good source of disease-resistant germplasm for eggplant (*Solanum melongena* L.) breeding. To facilitate novel genetic manipulation of this germplasm source and introgression of genes into other eggplant species this study established protocols for the rapid and efficient regeneration of plants in tissue culture. The growth response of hypocotyl, cotyledon and leaf explants of Cuzi scarlet eggplant was investigated on MS medium supplemented with various auxins and cytokinins and some selected combinations of auxins and cytokinins. Both hypocotyl and cotyledon explants were highly responsive for shoot regeneration within 20 days on MS medium supplemented with 2 mg l^{-1} to 4 mg l^{-1} 6-benzylaminopurine with or without 0.5 mg l^{-1} indole-3-acetic acid. All regenerated shoots could be easily induced to initiate roots within five to ten days following transfer to MS medium without growth regulators.

Keywords: adventitious regeneration - cuzi eggplant - organogenesis - scarlet eggplant - tissue culture - *Solanum aethiopicum.*

Introduction

Scarlet eggplant (*Solanum aethiopicum* L.) is cultivated throughout most of tropical Africa and some areas of Asia. Four distinct groups of scarlet eggplant cultivars are recognised as originating by domestication from a single wild progenitor, *S. anguivi* (Lester & Niakan 1986). These four groups, previously treated as separate botanical species, are now recognised by the names: Gilo, Shum, Kumba and Aculeatum (Lester 1986, Daunay *et al.* 1995). The Aculeatum group (usually referred to as *S. integrifolium*) is distinguished by pubescent mature leaves, very prickly stems and leaves, and numerous furrows on the fruit

(Lester & Niakan 1986). It is usually associated with being cultivated as an ornamental plant (Lester 1986, Lester & Niakan 1986). However, in the Yunnan province of China, populations of plants from this group are locally known as Cuzi and encouraged to grow in a semi-naturalised state to harvest the orange fruit for cooking as a vegetable.

Scarlet eggplant is also known to be a good source of disease-resistant germplasm for eggplant (*Solanum melongena* L.) breeding (Dauney *et al.* 1991). It has already been used in eggplant improvement as a source of resistance to bacterial wilt incited by *Ralstonia solanacearum* (Ano *et al.* 1991; Hébert 1985) and *Fusarium oxysporum* f. sp. *melongenae* (Cappellii *et al.* 1995). Cuzi is locally recognised as having high resistance against *Verticillium* wilt, and is often used as a rootstock for the grafting of eggplants as a means of protecting eggplants from root diseases (Lin & Xiao 1995).

Cell and tissue culture can be used in a wide range of applications for the genetic manipulation of plants. Rapid and efficient regeneration of shoots from cell cultures is important for applications such as embryo rescue following wide hybridisation, somatic cell selection, somatic hybridisation and transformation of foreign genes.

In this paper we investigate adventitious shoots regeneration from tissue culture explants of Cuzi scarlet eggplant. The intention is to provide a basis for novel genetic manipulation of this germplasm source and introgression of genes into other eggplant species.

Materials and Methods

Seeds of Cuzi scarlet eggplant were surface-sterilised for 5 seconds in absolute ethanol followed by 10 min in 1.5 % sodium hypochlorite (plus a drop of Tween 20). After washing three times in sterile distilled water, the seeds were germinated in 290 ml plastic pottles (80 mm diameter x 60 mm high; Vertex Plastics, Hamilton, New Zealand) on MS salts and vitamins (Murashige & Skoog 1962) plus 3 % (w/v) sucrose and 0.8 % (w/v) Gibco bacteriological agar with pH adjusted to 5.8.

Explants used for *in vitro* adventitious regeneration studies consisted of cotyledons cut in both dimensions to give four segments and hypocotyl segments (10 mm long) from 10-day-old *in vitro* seedlings, as well as leaf discs (about 100 mm^2) from 15-day-old *in vitro* seedlings. All explants were cultured in standard plastic Petri dishes (Biolab, Christchurch, New Zealand) on MS salts and vitamins (Murashige & Skoog 1962), supplemented with a range of plant growth regulators, plus 2 % (w/v) sucrose and 0.8 % (w/v) Gibco bacteriological agar with pH adjusted to 5.8. Plant growth regulator treatments involved 0.1-20 mg l^{-1} IAA (indole-3-acetic acid), IBA (indole-3-butryric acid) and NAA (α-naphthaleneacetic acid); as well as 0.1 to 4 mg l^{-1} BAP (6-benzylaminopurine), 2iP (N^6-(2-isopentyl) adenine), kinetin (6-furfurylaminopurine), TDZ (thidiazuron) and zeatin (6-(4-hydrozy-3-methylbut-2-enylamino) purine). Two combinations of plant growth regulators were also investigated based on previous use for successful adventitious regeneration of eggplant; 1 mg l^{-1} IAA plus 0.5 mg l^{-1} zeatin (Kamat & Rao 1978) and 0.5 mg l^{-1} IAA plus 2.5 mg l^{-1} BAP (Sharma & Rajam 1995).

All culture media were autoclaved for 15 min at 103 kPa, except filter sterilised zeatin was added just before media were dispensed into pre-sterilised

culture vessels. Ten explants were placed in each Petri dish with six replicate Petri dishes per treatment. All culture vessels were sealed with plastic wrap and incubated at 25 ± 2 °C under light from cool white fluorescent lamps (80-100 μmol m^{-2} sec^{-1}; 16 h photoperiod). Regenerated shoots were only counted when they could be excised as an intact shoot for transfer to a rooting medium.

Mean shoot numbers per explant for each dish were analysed with a Poisson generalised linear model, with a logarithmic link (McCullagh & Nelder 1989). Comparisons between the treatments were done using F-tests within the analysis of deviance carried out as part of the analysis. Results are presented as means with 95 % confidence limits, which were calculated on the log. scale, and then back transformed. Analyses were carried out using GenStat (GenStat Committee 2006).

Results

MS medium supplemented with auxins

IAA promoted root induction and completely inhibited the development of shoots. No growth response was observed from cotyledon and leaf explants without IAA. The addition of 0.1 - 1mg l^{-1} IAA promoted root development with no growth response at 2 mg l^{-1} IAA and above. In contrast, hypocotyl explants each produced 1 - 2 small shoots without auxins. Single small shoots were often observed with the addition of 0.1 mg l^{-1} IAA, but at concentrations of 0.5 - 1.0 mg l^{-1} IAA only root and callus growth was observed, with usually no growth response at 2 mg l^{-1} IAA. IBA resulted in a similar growth response to IAA, except the magnitude of response was less. All explants only developed callus with supplementation of the medium with 0.1 - 20 mg l^{-1} NAA.

MS medium supplemented with cytokinin

The culture of cotyledon and hypocotyl explants on MS medium supplemented with varying concentrations of BAP, 2iP, kinetin, TDZ and zeatin (0.1 to 4.0 mg l^{-1}) was tested in initial experiments. The growth response ranged from the superficial appearance of shoot primordial to regeneration of shoots,

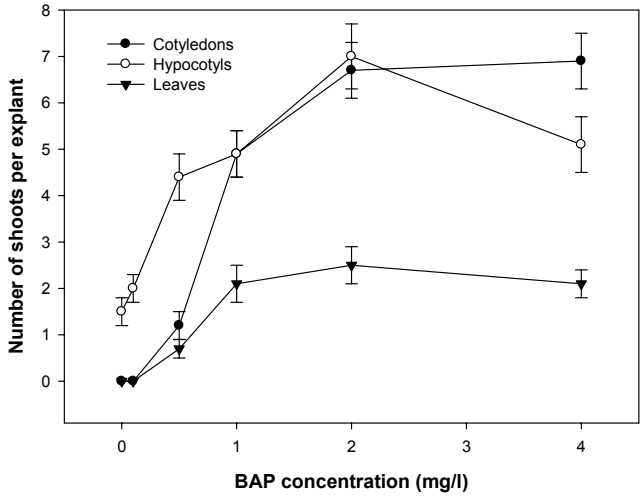

Figure 1. Mean number of adventitious shoots regenerated from each explant source after 20 days in response to varying BAP concentrations (with 95% confidence limits).

especially for hypocotyl explants. For 2iP, kinetin and TDZ it was often difficult to remove intact shoots from the explants, despite the presence of many shoot primordial. The development of well formed shoots was especially evident in the presence of BAP, and to a lesser extent for lower concentrations of zeatin.

The growth response to increasing concentrations of BAP was further assessed for cotyledon, hypocotyl and leaf explants (Figure 1). There was a significant interaction between BAP concentration and explant type ($P < 0.001$), indicating that the change in shoot numbers with increasing BAP level varied between the explants. Without BAP no shoots developed on the cotyledon or leaf explants, but just over one shoot developed per hypocotyl explant ($P < 0.05$).

In the presence of BAP the number of shoots increased with increasing BAP concentration up to 2 mg l^{-1} but at different rates. The rate of increase was greatest for the cotyledon explants, followed by the hypocotyl explants, with the rate of increase much less for leaf explants. Cotyledon and hypocotyl explants regenerated up to seven shoots per explant. However, numbers of shoots from hypocotyl explants decreased to five ($P < 0.05$) when BAP was increased from 2 to 4 mg l^{-1}. The number of shoots from leaf segments remained quite low, with a maximum mean of 2.5 shoots per explant at 2 mg l^{-1} BAP, significantly lower ($P < 0.001$) than for the cotyledon and hypocotyl explants at the higher BAP levels. All regenerated shoots could be easily induced to initiate roots within five to ten days following transfer to MS medium without growth regulators.

MS medium supplemented with auxin and cytokinin

Two media formulations commonly used for shoot regeneration in eggplant (*Solanum melongena*) are MS medium supplemented with 0.5 mg l^{-1} IAA plus 2.5 mg l^{-1} BAP (Sharma & Rajam 1995) and 1 mg l^{-1} IAA plus 0.5 mg l^{-1} zeatin (Kamat & Rao 1978). These plant

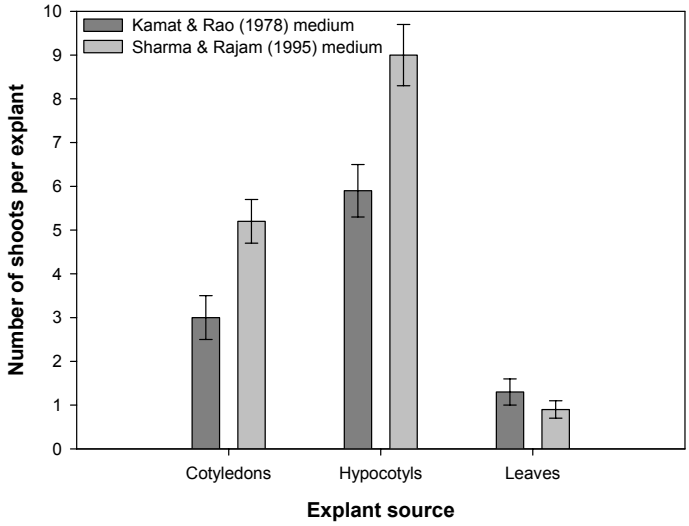

Figure 2. Mean number of adventitious shoots regenerated from each explant source after 20 days culture on the media formulations of Sharma & Rajam (1995) and Kamat & Rao (1978) (with 95% confidence limits).

growth regulators, at approximately these concentrations, induced the highest growth response when evaluated individually on Cuzi scarlet eggplant. Therefore, the performance of cotyledon, hypocotyl and leaf explants were compared on these two media formulations. On both media, cotyledon and leaf explants of Cuzi scarlet eggplant developed roots within one week. No roots were observed on the hypocotyl explants. Shoot development was initiated on all explants within 10 days, with well formed shoots counted after 20 days (Figure 2). There was a significant interaction between media and explant source ($P < 0.001$), indicating that the difference between the two media varied with explant type. For leaf segments the numbers of shoots were generally low with only ~1 shoot per explant, substantially lower than for the two other explant sources ($P < 0.001$), with little difference between the two media ($P = 0.08$). The numbers of shoots were generally higher from hypocotyl explants than from cotyledon explants ($P < 0.001$). For these two explant sources approximately 1.6 times more shoots regenerated on the Sharma & Rajam (1995) medium than on the Kamat & Rao (1978) medium. After a further 10 days culture, most regenerated shoots had grown to attain 2 - 4 leaves. During this period, some shoots also developed roots at their base. Following excision from the explants, all shoots initiated roots within five to seven days after transfer to MS medium without growth regulators.

Discussion

Adventitious organ development in tissues culture is well studied in *Solanum melongena* (eggplant) compared to other closely related species (Gleddie *et al.* 1983). Consequently, studies on *S. melongena* can provide a valuable guide to possible culture medium formulations and anticipated growth responses for species such as Cuzi scarlet eggplant (*Solanum aethiopicum*). In experiments on *S. melongena*, cytokinins such as BAP induce shoot bud primordia (Gleddie *et al.* 1983), whereas the use of auxins such as NAA induced numerous somatic embryos (Matsuoka & Hinata 1979; Gleddie *et al.* 1983). A similar response to cytokinins such as BAP was observed for Cuzi scarlet eggplant. Supplementing MS medium with 1 - 4 mg l^{-1} BAP resulted in the rapid development of multiple complete shoots from all explants sources of Cuzi scarlet eggplant. These shoots readily initiated roots when transferred to medium with plant growth regulators. In contrast to *S. melongena*, all explant sources of Cuzi scarlet eggplant failed to produce somatic embryos or embryogenic calli on all the culture medium investigated in this study. In earlier work, a high potential for somatic embryo production in *S. melongena* was associated with a low potential for adventitious root and shoot organogenesis and *vice versa* (Matsuoka & Hinata 1979). This appears to also hold true for Cuzi scarlet eggplant.

Efficient shoot regeneration in *S. melongena* is usually obtained on culture media with the auxin IAA combined with a cytokinin such as BAP or zeatin (Gleddie *et al.* 1983). Two commonly used media formulations are 1 mg l^{-1} IAA plus 0.5 mg l^{-1} zeatin (Kamat & Rao 1978) or 0.5 mg l^{-1} IAA plus 2.5 mg l^{-1} BAP (Sharma & Rajam 1995). A comparison of these two medium formulations for Cuzi scarlet eggplant demonstrated more regenerated shoots per explant were induced from the Sharma and Rajam (1995) medium for cotyledon and

hypocotyl explants. No difference between these two media was apparent for leaf explants, although only low numbers of shoots were regenerated from leaf segments.

In conclusion, this study has defined approaches for rapid adventitious regeneration of complete plants from tissue culture explants of Cuzi scarlet eggplant (*Solanum aethiopicum*). This therefore provides the basis for biotechnology approaches to genetic improvement of this species and the introgression of genes into other eggplant species.

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